

Population Genetics of the Sandbar Shark (*Carcharhinus plumbeus*) in the Gulf of Mexico and Mid-Atlantic Bight

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Allozyme electrophoresis and restriction fragment length polymorphism analysis of mitochondrial DNA (mtDNA) were performed on sandbar sharks (*Carcharhinus plumbeus*) from coastal waters of Virginia, including the Chesapeake Bay, and the Gulf of Mexico to test the hypothesis that individuals from the two locations comprise a single gene pool. Both techniques revealed a very low degree of genetic variability within the species (allozyme mean heterozygosity = 0.005, mean nucleotide sequence diversity = 0.036%). The small amount of genetic variation present appeared to be evenly distributed between sampling locations; and therefore, the null hypothesis of a single gene pool could not be rejected (contingency $\chi^2 = 1.344$, $P > 0.5$, chi-square significance of mtDNA haplotype distribution = 0.81). This conclusion is consistent with the known life-history characteristics of the species, as well as the results of tagging studies.

THE sandbar shark (*Carcharhinus plumbeus*) is a large (maximum adult length > 2 m), coastal species that has numerous allopatric populations throughout the warm temperate and subtropical oceans (Compagno, FAO, 1984, unpubl.). The species ranges from Massachusetts to Brazil along the western North Atlantic coast, including the Caribbean Sea (Compagno, FAO, 1984, unpubl.). The sandbar shark is the most common species of large shark in the United States' coastal Atlantic waters as well as the Gulf of Mexico (Springer, 1960) and is one of the most important sharks in the United States' shark longline fishery (Musick et al., 1993).

In the western North Atlantic, the sandbar shark undergoes seasonal migrations; however, the two sexes become segregated as adults. Male/female ratios are nearly equal at birth (Springer, 1960; Clarke and von Schmidt, 1965), but larger individuals captured in the mid-Atlantic Bight are almost exclusively female with larger males remaining to the south and farther offshore. The largest pupping grounds for the sandbar shark occur in the mid-Atlantic Bight from New York to Cape Hatteras and especially in Chesapeake Bay (J. A. Musick and J. A. Colvocoresses, unpubl.). Young sandbar sharks of both sexes are common in inshore regions of these latitudes during the summer but move offshore and southward during the winter, presumably to the edge of the Gulf Stream off North Carolina (Compagno, FAO, 1984, unpubl.). Larger individuals (age five years and older) migrate south to the Gulf of Mexico, as revealed by the dozens of individuals tagged in the mid-Atlantic Bight that were later recaptured in the Gulf of Mexico (Casey and Kohler, 1990).

Movement of individuals throughout a pop-

ulation's range does not preclude genetic divergence within the putative population. Springer (1960) suggested that the sandbar sharks of the western North Atlantic may contain two separate breeding populations, a major one off the mid-Atlantic coast of North America and a minor one in the western Gulf of Mexico. If Springer's suggestions are correct, then the sandbar sharks that occupy the Gulf of Mexico may include a mixture of stocks from both pupping grounds.

Differences in the migrational tendencies of males and females may have a differential effect on mitochondrial genes vs nuclear genes. If female sandbar sharks have separate natal pupping zones in the western North Atlantic, it is possible that mtDNA haplotype frequencies have diverged between pupping locations regardless of the movements of the males. Meylan et al. (1990) found that, although green sea turtles from various natal beaches have overlapping feeding grounds, frequencies of mtDNA genotypes differed greatly between females from different natal islands. Karl et al. (1992) showed that Mendelian characteristics in green turtles showed less divergence, indicating male-mediated gene flow. The Gulf of Mexico may contain mixtures of sandbar sharks from various pupping areas; and, therefore, haplotypes common in other regions (e.g., the Caribbean Sea) may be present at low frequencies within the Gulf of Mexico but completely absent in the Chesapeake Bay. Biparental inheritance of allozyme characters will prevent divergence if even small number of males (i.e., > one per generation) mate with females from other pupping grounds (Wright, 1978).

Several studies have used molecular tech-

niques to analyze population genetics of temperate and subtropical marine fishes between the Gulf of Mexico and the Atlantic coast of North America (reviewed by Avise, 1992). The subtropical southern point of the Florida peninsula today serves as a barrier to gene flow between populations of many temperate species of marine fishes and invertebrates. Some species with limited migrational tendencies, (e.g., horseshoe crab, black sea bass) exhibit genetic divergence between the Gulf of Mexico and the southeastern coast of the United States (Saunders et al., 1986; Bowen and Avise, 1990), whereas other highly migratory species of fishes (e.g., American eel, bluefish) display no significant genetic divergence (Avise et al., 1986; Graves et al., 1992). During the heights of the Wisconsin glaciation, ending 10,000–15,000 yr before present, temperate populations that are now subdivided may have mixed in a refugia in the Caribbean Sea or southern Gulf of Mexico. Current levels of genetic divergence may have been influenced by both current patterns of gene flow and historical differences in species distribution. Our study uses both allozyme electrophoresis and RFLP analysis of mtDNA to test the null hypothesis that sandbar sharks from the Gulf of Mexico and the Chesapeake Bay and adjacent coastal waters represent a single gene pool.

METHODS

Sample collection.—Sandbar sharks were captured with research longlines from the Chesapeake Bay and adjacent coastal waters of Virginia ("Bay"), $n = 173$, as part of the ongoing shark research program of the Virginia Institute of Marine Science (VIMS). Sharks were also collected from the Gulf of Mexico along the southwestern coast of Florida ("Gulf"), $n = 222$, aboard a commercial shark fishing vessel and from artisanal longline vessels from Veracruz, Mexico, $n = 5$, (Fig. 1). The temporal sampling strategy involved the collection of Bay specimens separately during three consecutive summers, 1990–1992; Florida Gulf specimens in Feb. and Sept. 1991; and Mexican Gulf samples in April 1993. The majority of the Bay specimens were juveniles less than 1 m total length and were presumably less than five years of age. These sharks were apparently too young to have made a migration to the Gulf of Mexico (Casey et al., 1985). The Gulf samples were from adult and subadult sharks greater than 1.5 m total length. One individual captured in the Gulf of Mexico in 1991 had been tagged in the Atlantic off Montauk Point (J. Casey, cooperative shark

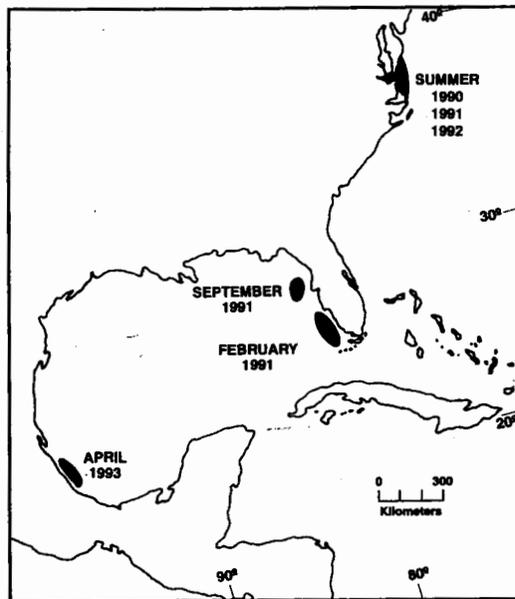


Fig. 1. Dates and locations of sample collections for the sandbar shark.

tagging program, pers. comm.). Heart, kidney, and white muscle tissue from each of the specimens were removed and frozen immediately in liquid nitrogen, except for the Mexican samples that were collected as whole hearts, chilled on wet ice, and temporarily stored in a household freezer (-20°C) for five to 10 days. After transport to the lab, tissues were stored at -70°C until analyzed.

Allozyme electrophoresis.—Homogenates were separately prepared with heart, kidney, and white muscle tissues by grinding with a mechanical tissue homogenizer in approximately two volumes of ice-cold grinding buffer (100 mM tris, 1 mM EDTA, pH 7.0). The resultant preparation was centrifuged at 1200 g for 10 min, and the supernatant decanted and stored at -70°C prior to electrophoresis. The homogenates were subjected to electrophoresis on 12% horizontal starch gels (Starch Art, Inc.) using the apparatus of Murphy et al. (1990) for 16–20 hr at 2.5 V/cm. Extracts of each tissue type were separated electrophoretically on eight different buffer systems and stained with approximately 40 histochemical stains to determine a suite of tissue/buffer/stain combinations that adequately resolved products of a set of presumptive allozyme loci (Table 1). All buffers and histochemical stains followed Murphy et al. (1990) except for the triethanolamine-citrate (TRIC) buffer of Clayton and Tretiak (1972).

TABLE 1. ENZYMES AND LOCI RESOLVED, AND THE NUMBER (N) OF INDIVIDUALS ASSAYED IN THE SANDBAR SHARK.

Enzyme (Enzyme Commission Number) ^a	Locus	Tissue ^b	Buffer ^c	Sample size	
				Bay	Gulf
Aconitate hydratase (4.2.1.3)	Acon-A	H	TCIII	36	66
Adenylate kinase (2.7.4.3)	Ak-2	M	TCII	36	70
Creatine kinase (2.7.3.2)	Ck-C	H	TE	42	72
Dihydrolipoamide dehydrogenase (1.8.1.4)	Ddh-A	K	TCIII	39	64
Esterase (3.1.1.-)	Est-1	K	Borate	54	74
Esterase (3.1.1.-)	Est-2	K	Borate	57	74
Esterase (3.1.1.-)	Est-3	K	Borate	57	74
Formaldehyde dehydrogenase (1.2.1.1)	Fdh-A	K	TRIC	36	74
Glycerol-3-phosphate dehydrogenase (1.2.1.8)	G3dph-A	M	TCIII	40	66
Glucose-6-phosphate dehydrogenase (1.1.1.49)	G6dph-A	K	TCIII	36	70
α -Glucosidase (3.2.1.20)	α Glus-A	K	TCIII	36	68
L-Iditol Dehydrogenase (1.1.1.14)	Iddh-A	K	TCIII	31	73
L-Lactate dehydrogenase (1.1.1.27)	Ldh-A	H or K	TRIC	173	222
L-Lactate dehydrogenase (1.1.1.27)	Ldh-B	H or K	TRIC	173	222
Malate dehydrogenase-NAD (1.1.1.37)	mMdh-A	H	CAPM	42	70
Malate dehydrogenase-NAD (1.1.1.37)	sMdh-B	H	CAPM	42	70
Malate dehydrogenase-NADP (1.1.1.40)	mMdhp-A	H or M	CAPM	42	74
Malate dehydrogenase-NADP (1.1.1.40)	sMdhp-A	H or M	CAPM	42	74
α -Manosidase (3.2.1.24)	α Man-A	K	TE	65	57
Octanol dehydrogenase (1.1.1.73)	Odh-A	K	TE	54	67
Peptidase (3.4.-.-)	Pep-1 ^d	K	LiOH	54	64
Peptidase (3.4.-.-)	Pep-2 ^d	K	LiOH	54	52
Peptidase (3.4.-.-)	Pep-3 ^d	K	LiOH	54	52
Peptidase (3.4.-.-)	Pep-4 ^d	K	LiOH	54	59
Phosphogluconate dehydrogenase (1.1.1.44)	Pgdh-2	K	TCIII	54	49
Superoxide dismutase-1 (1.15.1.1)	Sod-1	H	CAPM	36	64
Superoxide dismutase-2 (1.15.1.1)	Sod-2	H	CAPM	36	64

^a IUBMBNC (1992).

^b H = heart, K = kidney, M = white muscle.

^c (a) Borate (continuous) = pH 8.6, (b) CAPM = Citric acid Aminopropyl morpholine, pH 6.0, (c) LiOH = Lithium-borate/tris citrate, pH 8.3, (d) Tris-citrate II, pH 8.0, (e) Tris-citrate III, pH 7.2, (f) Tris-EDTA, pH 9.6.

^d Four zones of peptidase activity were observed using three peptide substrates. Presumptive loci are listed in order of increasing anodal mobility (Pep-1 = Leu-Pro, Pep-2 = Leu-Gly-Gly, Pep-3 = Leu-Gly-Gly, Pep-4 = Ala-Met).

Wright's F statistics (Wright, 1978) and chi-square analysis of Hardy-Weinberg expectations were calculated using the BIOSYS-1 program of Swofford and Selander (1981).

Mitochondrial DNA RFLP analysis.—Mitochondrial DNA was isolated from heart tissue using cesium chloride density-gradient ultracentrifugation following Lansman et al. (1981). Aliquots of mtDNA were digested using 12 restriction enzymes following the manufacturers' instructions. Fragments were separated on 1.0% horizontal agarose gels run at 2V/cm overnight and visualized following one of two techniques. When tissue was abundant and freshly frozen, mtDNA fragments were radiolabeled with ³²S-labeled nucleotides prior to electrophoresis using the large (Klenow) fragment of DNA polymerase I (Sambrook et al., 1989). After electrophoresis, the gels were treated with a scin-

tillation enhancer, dried, exposed to x-ray film for 3–6 days, and visualized autoradiographically. For those specimens with small tissue samples or with partially degraded tissue, fragments were transferred after electrophoresis to a nylon membrane via Southern transfer following the protocols of Sambrook et al. (1989). The fragments were probed with highly purified shortfin mako (*Isurus oxyrinchus*) mtDNA nick-translated with biotin-7 dATP and visualized using the BRL BlueGene Nonradioactive Nucleic Acid Detection System.

Fragment patterns were scored for each restriction enzyme, and each individual was assigned a composite genotype based on the fragment patterns for all enzymes. Each polymorphism (RFLP) could be explained by the gain or loss of one (or in one case two) restriction sites relative to the common pattern. The nucleon (genotypic) diversity was calculated for



Fig. 2. Zymogram of lactate dehydrogenase (LDH) expression from heart extracts of sandbar shark. Specimens 1, 4, 6, and 8 are 100/100 homozygotes for both the A and B loci. Specimens 3, 5, and 7 are 100/90 heterozygotes for the B locus, and homozygotes (100/100) for the A locus. Specimen 2 is a 90/90 homozygote for the B locus and a 100/100 homozygote for the A locus.

each sample and for the composite of both samples following Nei (1987). Nucleotide sequence diversity was calculated following the site approach of Nei and Li (1979). Chi-square significance of the difference in genotypic frequencies between samples was computed using the randomization protocol of Roff and Bentzen (1989).

RESULTS

Allozyme electrophoresis.—Products of 27 presumptive loci were scored for a minimum of 100 individuals (Table 1). Products of approximately 20 other presumptive loci were observed in a smaller number of individuals but were not resolved clearly. Twenty-five of 27 loci were monoallelic in all samples. Overall 3.58% of the loci assayed were variable, with an average heterozygosity among loci and samples equal to 0.005.

Only one locus, *Ldh-B*, was sufficiently polyallelic to be used to test population structure hypotheses (Fig. 2). A second locus, *mMdh-A*, produced a single heterozygote in 116 individuals. Aspartate transaminase (*sAta-A*) appeared to have a high degree of heterozygosity but was one of the loci excluded from the analysis because of poor resolution.

The overall frequency of the most common *Ldh-B* allele was 0.928, and frequencies were very similar for the Bay (0.922) and Gulf (0.932) samples. Observed *Ldh-B* genotypic frequencies conformed to expected Hardy-Weinberg proportions in both the Bay ($\chi^2 < 0.001$, $P > 0.98$) and Gulf ($\chi^2 = 1.125$, $P > 0.25$) populations. A contingency chi-square analysis indicated that the observed genotypic distributions

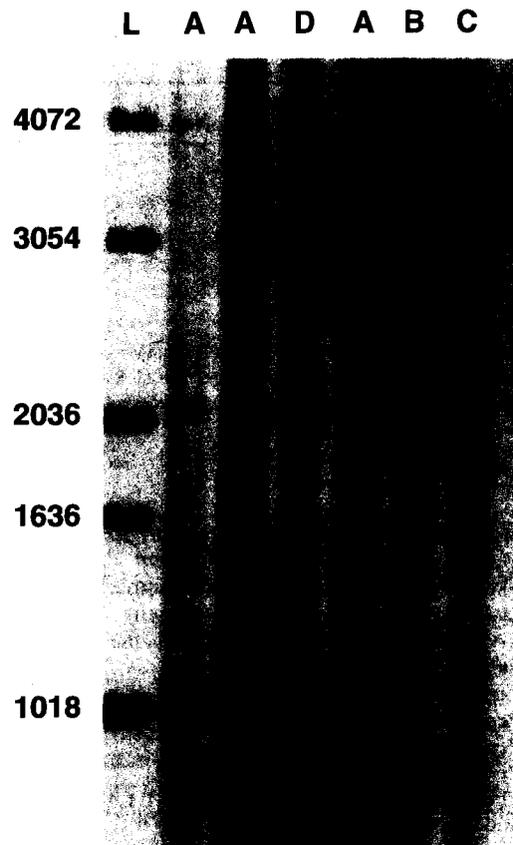


Fig. 3. Mitochondrial DNA restriction fragment patterns for *Ava* II. Lane L is a 1-kilobase (kb) size standard, numbers on left correspond to fragment sizes (base pairs) of standard. Missing from each sample lane is one 6.05 kb band and one to two bands less than 0.4 kb. See Table 3 for fragment sizes.

did not differ significantly from the sampling of a single population with an allele frequency of 0.928 ($\chi^2 = 1.344$, $P > 0.5$).

Analysis of mitochondrial DNA.—Mitochondrial DNA restriction patterns were determined for 95 sandbar sharks using 12 restriction enzymes (Fig. 3; Table 2). Ten of 12 restriction enzymes revealed invariant fragment patterns in all individuals. The other two restriction enzymes yielded a total of five haplotypes (Table 3), four of which occurred at very low frequency (< 0.04) and differed from the common pattern by one or two restriction sites. Not all individuals were scored for all enzymes; 3.7% of the cells in the restriction fragment pattern matrix were unscorable because of the exhaustion of tissue samples. However, individuals with missing restriction pattern data were assumed to possess the common pattern only for those enzymes in which no variants were detected after scoring

TABLE 2. ESTIMATED SIZE IN KILOBASE PAIRS (kb) OF mtDNA RESTRICTION FRAGMENTS FOR VARIOUS RESTRICTION ENZYMES. Letter in parentheses refers to fragment pattern designation.

Ava I	Ava II				Ban I
	(A)	(B)	(C)	(D)	
5.48	6.05	6.05	6.05	6.05	7.10
5.27	2.20	2.20	2.20	2.20	5.65
2.44	2.20	1.86	2.20	2.20	4.52
1.38	1.60	1.60	1.60	1.60	17.27
1.13	1.09	1.09	1.55	1.09	
0.95	1.09	1.09	1.09	1.00	
16.65	0.87	0.87	0.87	0.87	
	0.77	0.77	0.77	0.77	
	0.63	0.63	0.63	0.63	
	0.30	0.30	16.96	0.30	
	0.20	0.30		0.20	
	17.00	0.20		16.91	
		16.96			
Bcl I	Bgl I	Dra I	Hae II	Hinc II	
6.75	12.00	5.65	9.50	3.76	
4.18	4.71	4.81	7.00	3.42	
2.24	16.71	2.32	16.50	3.23	
1.84		1.93		1.68	
1.30		1.52		1.48	
0.95		16.23		0.93	
17.26				0.80	
				0.61	
				15.91	
Hind III					
(A)	(B)	Nru I	Sca I	Xho I	
4.69	4.69	12.00	9.50	9.00	
3.55	3.55	4.50	6.07	5.31	
3.28	3.28	16.50	1.12	2.35	
2.69	2.99		16.69	16.66	
1.34	1.34				
0.92	0.92				
0.30	16.77				
16.77					

a minimum of 85 individuals. The approximate size of the sandbar shark mitochondrial molecule was estimated at 16.7 kilobase pairs (Table 2). Twelve restriction enzymes with four-, five-, and six-base recognition sites produced an average total of 58 restriction fragments per individual. The mean number of bases surveyed was 301, or 1.79% of the mitochondrial DNA molecule. The composite nucleon diversity was very low (0.161) with 87 of 95 individuals sharing the common genotype (Table 3). The Gulf sample had a slightly higher nucleon diversity (n.d.) than the Bay sample (n.d. = 0.22 and 0.11, respectively).

The corrected mean nucleotide sequence divergence between the Bay and Gulf samples was

TABLE 3. MITOCHONDRIAL RESTRICTION PATTERNS OBSERVED, GENOTYPES DESIGNATED, NUMBER OF GENOTYPES OBSERVED IN THE SANDBAR SHARK (*Carcharhinus plumbeus*). Enzymes scored (left to right) are Ava I, Ava II, Ban I, Bcl I, Bgl I, Dra I, Hae II, Hinc II, Hind III, Nru I, Sca I, Xho I.

Genotype	Pattern	N				
1	AAAAAAAAAAAA	87				
2	ABAAAAAAAAAAAA	3				
3	ACAAAAAAAAAAAA	2				
4	ADAAAAAAAAAAAA	2				
5	AAAAAAAAABAAA	1				
Total	95					
Nucleon diversity = 0.161						
Chesapeake Bay						
Time of Collection	Genotype					Total
	1	2	3	4	5	
Summer 1990	14	0	1	0	0	15
Summer 1991	21	1	0	0	0	22
Summer 1992	14	0	0	1	0	15
Total	49	1	1	1	0	52
Gulf of Mexico						
Location/Time of collection	Genotype					Total
	1	2	3	4	5	
Florida, Feb. 1991	13	2	1	0	0	16
Florida, Sep. 1991	20	0	0	1	1	22
Veracruz, April 1993	5	0	0	0	0	5
Total	38	2	1	1	1	43

less than 0.001%, compared to the within sample nucleotide sequence diversities of 0.026% for the Bay sample and 0.046% for the Gulf sample. The overall mean nucleotide sequence diversity was 0.036%. Of the five mitochondrial genotypes detected, four were encountered in both samples; and the remaining genotype was present in only a single individual in the Gulf sample (Table 3). All five individuals from Veracruz possessed the common genotype and were, thus, grouped with the Florida samples into a common Gulf sample. The Roff and Benzen (1989) randomization analysis produced a chi-square value higher than the observed value in 812 of 1000 randomizations of the genotype frequency data, suggesting that the null hypothesis of a single gene pool could not be rejected ($P = 0.81$).

DISCUSSION

Sandbar sharks are characterized by relatively low levels of genetic variation. Smith (1986) reported a range of allozyme heterozygosities of 0.0011–0.037 for seven species of sharks, with the highest heterozygosity belonging to blue shark, (*Prionace glauca*), the only carchar-

hinid in that study. Lavery and Shaklee (1989) reported heterozygosities of 0.035 and 0.037 for two species of *Carcharhinus* in Australia. The heterozygosity of 0.005 determined for *C. plumbeus* in this study is nearly an order of magnitude less than that of the above published accounts of heterozygosity in carcharhinids and is low compared to typical values for other fishes (see summary in Smith and Fujio, 1982). The nucleon diversity of 0.16 and the nucleotide sequence diversity of 0.036% for the sandbar shark are very low compared to the numerous values published for teleost taxa (Avisé et al., 1989; Ovenden, 1990).

Neither allozyme nor mtDNA analysis provide evidence to reject the null hypothesis that the samples share a single gene pool. Although genetic variability was extremely low for both allozymes and mtDNA, the similar frequencies of Ldh-B alleles and the occurrence of the same rare mtDNA alleles in both locations supports the hypothesis that samples were collected from a single population. No evidence of the contribution to the stocks from pupping grounds with different genetic characteristics was detected.

Although populations of large coastal sharks, including sandbar sharks, have precipitously declined over the last decade because of the great increase in commercial shark exploitation, numbers of young sandbar sharks remain relatively high in the Chesapeake Bay (Musick et al., 1993). This apparent contradiction to the parent-stock recruitment relationship may be the result of reduced predation on young sandbar sharks by the relative scarcity of adults of larger species (Musick et al., 1993).

Overexploitation of large sharks can have severe and long-lasting effects on shark populations because of the slow growth rate, high age at maturity, and low fecundity of large, viviparous sharks (Holden, 1974; Hoff and Musick, 1990). As catches of sharks decline in the offshore waters of the Gulf of Mexico and mid-Atlantic Bight, commercial fishers have sought access to sharks within the nearshore coastal waters under state jurisdiction. The results of this study indicate that the young sandbar sharks of the coastal waters of Virginia are members of the same stock that has declined in number in the Gulf of Mexico and Atlantic coastal waters of the United States. Thus, further exploitation of these young sandbar sharks may have impacts on the future availability of the species as far away as the Mexican coast.

Low heterozygosities and genotypic diversities reduce the ability to test population genetic hypotheses without large sample sizes. Perhaps a technique that surveys a more variable region

of the sandbar shark genome, for example direct sequencing of mtDNA or examination of microsatellite DNA (e.g., Hughes and Queller, 1993), may be more appropriate. Martin et al. (1992) have suggested that a low rate of mtDNA evolution is typical in sharks. However, RFLP analysis of shortfin mako (*Isurus oxyrinchus*) mtDNA currently being performed in our laboratory indicates that low mtDNA variability is not universal in sharks; the shortfin mako has a considerably higher degree of genotypic and nucleotide sequence diversity.

Sandbar sharks in the western North Atlantic may, in fact, possess population structure that has not yet resulted in genetic divergence. During the Wisconsin glaciation, western Atlantic sandbar sharks may have been confined to a single refugia in the lower Gulf of Mexico and Caribbean Sea. Since the seas warmed and the range of the species extended northward, there may not have been enough time for stochastic processes to produce different frequencies of allozyme and mtDNA alleles between populations. This is possible because of the low degree of genetic variation and slow rate of molecular evolution (Martin et al., 1992) of this species.

The characterization of the allozyme and restriction fragment patterns of the sandbar shark in the western North Atlantic can provide a baseline for evaluating the structure of the species on a cosmopolitan scale. The sandbar shark's unusual distribution pattern—that of multiple isolated coastal populations separated by deep oceans and temperature regimes unfavorable to the species—make it a desirable species for testing the effects of gene flow between populations that are allopatrically remote. Future analysis of sandbar shark populations may provide additional information on potential levels of genetic divergence within patchily distributed species.

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Systematics of the Genus *Alopias* (Lamniformes: Alopiidae) with Evidence for the Existence of an Unrecognized Species

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Allozymic variation for 13 presumptive loci was used to infer phylogenetic relationships among geographic samples of thresher sharks of the genus *Alopias*. The existence of an unrecognized species of *Alopias* was suggested by the allozymic data. Trees were constructed from allozymic data using maximum-parsimony and Distance Wagner procedures. The coding procedure used in maximum-parsimony analysis united all samples of *A. superciliosus* and *A. pelagicus* into a single Operational Taxonomic Unit (OTU). Maximum-parsimony analysis identified a single most parsimonious tree that united the unrecognized species of *Alopias* and the *A. superciliosus* + *A. pelagicus* OTU into a clade excluding *A. vulpinus*. The Distance Wagner analysis united samples of *A. vulpinus* into a clade excluding samples of all other species; the unrecognized species of *Alopias* and *A. vulpinus* constituted a clade; and samples of *A. superciliosus* and *A. pelagicus* were united into a clade. Analyses of maximum-parsimony and Distance Wagner trees using FREQPARS showed that the maximum-parsimony tree was shorter. Some uncertainty regarding phylogenetic placement of the unrecognized species remained because the synapomorphy uniting this species into a clade with *A. superciliosus* and *A. pelagicus* was scorable only in a single specimen of the unrecognized species. Allozymic data were not sufficient to accurately determine relationships among different geographic samples of *A. superciliosus* and *A. pelagicus*. This may have resulted from the low number of specimens and loci examined and/or the high genetic similarity of these two species. A high proportion of potentially informative polyallelic loci was found within *Alopias*; however, the outgroup (*Isurus oxyrinchus*) did not possess character states found among ingroup forms for many of these loci. Allozymes do show promise for investigating phylogenetic relationships of sharks at the genus/species level provided the number of species (OTUs) is relatively low, an informative outgroup is used, and the high proportion of potentially informative loci found in this study is typical of other genera.

COMPAGNO (1984) recognized three species in the family Alopiidae (thresher sharks), all in the genus *Alopias*. Members of this family have a greatly elongated upper lobe

of the caudal fin that is used to herd and stun prey (Castro and Huber, 1992). The species include *A. pelagicus* (pelagic thresher), *A. vulpinus* (thresher shark), and *A. superciliosus* (bigeye